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Development and Maturation of the Lung:
Age Specific Vulnerabilities

(Slide 2)

I'm going to speak about the importance of early life environmental exposures on the respiratory health of children. It really addresses three of the seven questions that speakers were sent; the time periods in development that may be important; issues that relate to early life events that have implications for the health as people grow into adulthood; and finally, are there markers that we can use for susceptibility. I'll suggest right now that birth weight distributions, as you'll see, may be a very useful, easily-obtainable marker.

(Slide 3)

I'll begin by providing a bit of perspective. The slide indicates the way in which I have organized my presentation. I use asthma as a paradigm for airways obstructive disease in children. Severe asthma is characterized by low levels of lung function and a high degree of airways reactivity. For those of you not familiar with the latter term, airways reactivity is a characteristic of the air tubes of the lung to hyper-respond, if you will, to certain pharmacologic and environmental stimuli.

It turns out that these pulmonary factors that are characteristic of obstructive childhood lung disease are also those factors which are associated with the persistence of these diseases into adulthood. That is, the more severe the asthma in a child, as marked by impaired pulmonary function and degree of airways reactivity, the greater the likelihood that the disease persists into adult life. And even when one looks across populations, if you look at just the distribution of lung function in people without obvious disease, lung function in childhood is a good predicator of risk for obstructive lung disease in adulthood, as is high degree of airway reactivity. I'm going to come back to this point

(Slide 4)

What I'd like to do first is to provide a brief overview of the fetal development of the respiratory system. I'm going to focus on this, because really I think this is where some of the most interesting and exciting potential places to look are. This slide is from some work from Thurlbeck. I want to focus your attention on two critical periods.

One of these periods is called the pseudoglandular period, from about six to 16 weeks of gestation. It is during this period of time that virtually the entire conducting or airway system of the lung is laid down, and perhaps the beginnings of the system that's going to provide the air exchange portions of the lung.

The second critical phase is called the canalicular phase, which is from 16 to 28 weeks. Although not listed here as major events, the differentiation of the pulmonary epithelial cells and the appearance of the cells that produce the surface-active chemicals that help the lung essentially stay expanded when the fetus is born, appear towards the end of this period.

The rest of the development period, not that it's unimportant, really is one of progressive division of the airways and the formation of the final elaboration of the conducting system.

(Slide 5)

This is shown in this next slide. The important point here is that the structures called respiratory bronchioles, which are the last elements of the purely conducting part of the lung, before you get to the gas exchange part of the lung, are by in large complete somewhere between the 15th and 20th week of gestation. The remainder of the development of the lung in the second and third trimesters relates to progressive segmentation of the portions of the lung that will eventually lead to the air exchange portions of the lung.

(Slide 6)

This slide (#6) looks at the surface area in relation to growth. I put this here is just to make one point. Conventionally, we are used to assessing the progress of the pregnancy in terms of "weeks of gestation" and weight of the newborne. In fact, the development of the lung, especially its surface area and ultimately its gas exchange capabilities, is more linked to the lengthening of the fetus *in utero*. However, the peak period of fetal lengthening actually occurs at the end of the second trimester/beginning of the third trimester, whereas the most rapid period of weight gain actually takes place in the third trimester. So there is an important disconnect between the morphologic development of the fetus and the lung from the conventional ways that we assess fetal development.

Although I'm going to focus almost all of the subsequent talk when I get to looking at environmental effects, on the air tubes of the lung, since most obstructive lung diseases, particularly childhood asthma which is the most important of them, are evaluated; I don't want to forget that the major purpose of the lung is for gas exchange. Of course, the gas exchange portion of the lung has a different developmental time course than does the airways.

(Slide 7)

This slide (#7) shows a counting technique -- the numbers are not as important as the shape here and the timing. This looks at the development of gas exchanging areas between 18 weeks gestation and birth. You will notice that development begins at about 18 weeks and takes off fairly quickly.

(Slide 8)

About 85% of the alveoli, or the gas exchanging part of the lung, develop post-natally. Only about 15% are present on average when the baby is born. Most of this alveolar development is finished by two or three years of age.

So when looking at age-specific vulnerabilities in development of airways, really the big focus I think is the fetus. When one is talking about those factors which affect the volume and gas exchange part of the lung you're looking at the latter part of fetal life and the first year or two of life predominantly.

(Slide 9)

I've taken this from a chapter Mary Ellen Wohl wrote in a textbook on pediatric lung disease. She identifies here, factors that determine flow of air through lungs in the first year of life. I've underlined the ones she has identified as essentially changeable, or potentially under the influence of a variety of environmental factors.

Tube elastance is another way of saying how elastic is the structure, how likely is it to stay open when subject to forces. The physical properties of the airway wall and the lung are certainly changeable characteristics that determine the actual flow. Other factors that affect flow are the area at the alveolus and the site where flow is limited in a normal lung. We're going to focus a lot of the discussion on measures which basically evaluate the geometry, and indirectly the physical characteristics of the airway wall in assessing some environmental effects on the lung.

(Slide 10)

Finally these are just some data just to illustrate the point about what happens to lung function over the lifetime of people. This measure is length-corrected FEV-1 which, for those of you not familiar with these terms, is the amount of air you can blow out in one-second with a forced expiration. It's kind of a mixed measure of the mechanical characteristics of the lung.

This figure represents about a thousand datapoints from 290 asymptomatic males who were followed beginning at various ages for about 13 years. You will notice that there's a very sharp increase in the length-corrected growth of FEV-1 over the ages of about five to 20. There is this approximate plateau period, and then somewhere in the third and fourth decades you begin to see this slow decline, which is characteristic of the aging lung. So this period of childhood (starting at age five) and early adolescence is a period of very rapid growth of the entire respiratory system.

(Slide 11)

Now I'm going to focus on the first three factors (listed in slide 11) all of which have been identified as important determinants of both the occurrence and severity of childhood respiratory morbidity. We can use asthma as the paradigm. Equally important, for all of these factors, their levels in childhood turn out to be very strongly predictive, at a population level, of risks for various kinds of adult obstructive lung disease. Therefore, they are all useful for looking at the relationship between what happens between childhood and adulthood. I've simply identified respiratory symptoms and specific illnesses that are often studied as a way of assessing the impact of the first three factors on what individuals perceive: wheezing, cough, acute respiratory illnesses, and then of course asthma.

Clearly, infections are a major part of the environment, but I'm not going to have anything to say about it in the context of this talk.

(Slide 12)

I'm going to focus my attention initially on tobacco smoke, because I think it's a very good model and will offer some insights about where we might look. I suggest that some of what we've learned about

environmental tobacco smoke's effect on the lung in childhood and in early adolescence may very well be relevant ambient air pollutants.

Then I'm going to step out a little bit from the just purely anatomical development to the lung to talk a little bit about the development of fetal and early-life response to aero-allergens. This is highly relevant to respiratory tract morbidity.

(Slide 13)

This is from a study of a little more than 300 healthy, pre-term infants, all of whom were delivered prior to 36 weeks gestation (an average of 33 weeks) with average birth weights around 1800 to 2000 grams. The two measures are listed here. This first one, a measure of flow, is sort of an integrated measure of the both elastic properties, or the compliance properties of the air tubes and their resistance properties. You'll notice those infants were studied in the infant nursery, so they were not exposed to any tobacco smoke prior to leaving the hospital. You'll also notice that those infants whose mothers smoked, had considerably lower measures of these flows than infants whose mothers had not smoked.

The measure $T_{PTEF}:T_E$ is a measure of the integration between the mechanisms that link inspiration and expiration in infancy, which are different than in mature adults. In this case, a smaller number is worse than a higher number. You'll notice that there is also a considerable difference.

The important point here is that these measures which mark various characteristics of the mechanical behavior of the infant respiratory system are clearly determined prior to or very early in the third trimester. In fact, prior to, because many of these babies were less than 30 weeks of gestation.

So here are characteristics that predominantly have to do with the behavior of airways and with higher levels of integration of the mechanics and other mechanisms of respiratory control which have already been affected fairly early in pregnancy.

(Slide 14)

This next slide comes from some work in which I was involved. These data are from females on whom we made the same flow measurements that you saw before. This slide looks at the effects of *in utero* smoking. These children were measured at an average age of two weeks. You will notice the lower line of the children who were the girls whose mothers smoked; the upper line is the line of the girls whose mothers did not smoke during pregnancy. The parallelism of the lines is partly an artifact of the model, but I put it in here to illustrate -- and I'll show you some more data on this -- that there's clearly a tracking on the distribution of lung function that occurs very early in life. This has been observed in studies with adolescent children, in people who have been in populations that have been studied over 30 years of time between adolescence and childhood.

(Slide 15)

This is from a study that was done in Tucson. There were approximately 150 infants who were studied before the age of one year, and all of their respiratory function studies were done at a time when the infants had never experienced any respiratory symptomatology.

You will notice that in those children who subsequently developed transient wheezing episodes (i.e., they stopped wheezing before age 3) after their testing was done, and those who had persistent wheezing (that is, they were wheezing before age one and still wheezing at age six), their antecedent lung function was different (lower) than for those children who never wheeze. In other words, there was something different about them before they ever got sick. So the symptoms are not the cause of the functional abnormality.

These 500 children were restudied at age six years, and broken down into these classifications. Those children who wheezed very early in life but were no longer wheezing and not asthmatic, had a functional profile that was very similar to those who were persistent wheezing, most of whom were asthmatics. So this is just to illustrate again this notion of each tracking of the function of the respiratory system.

(Slide 16)

One of the things that we looked at in the study that I was involved in was if the hypothesis was that the effects of tobacco smoke were on airways of the lung. Then if we made measures in these children that affect the airways, which is the measure V_{FRC}, and we made measurements that look, if you will, at the volume characteristics of the lung, which is the measure FRC, in these children, the measures of their pulmonary function took place prior to the time any of them were ever sick -- you'll notice that the volume measures are not terribly different between those who developed wheezing illnesses and those who didn't. In the case of the flow measures (V_{FRC}), those children who subsequently had wheezing episodes actually had lower level of function than the kids who never wheezed.

So the effect appeared relatively specific to airway effects and not to volume effects, which is consistent with a lot of what we would have expected knowing that wheezing in the first year or two of life is a marker for subsequent respiratory disease.

(Slide 17)

The last couple slides are on environmental tobacco smoke. These are some data from the six-city study which looked at the effects of maternal smoking at various points in life. The important point of this slide is that when the investigators partitioned the exposure from age zero to five years, six to 10 years, 11 to 18 years, and if you reclassify the exposure at six to 10 years and 11 to 18 years, the effects of the exposure classification the first five years are as large as the current smoking status of the mother. So the point of this study was that there were effects essentially that had taken place early in life -- and they don't distinguish between smoke exposure *in utero* and postnatal; it's all lumped together – however, these effects that are measured here are independent of the persistent smoking behavior of the mother. Therefore, they represent early effects which are being carried forward.

Now, how do these observations relate to the environmental exposures that are more at issue here, particularly ambient air pollution exposures and potentially other toxicant effects upon the lung?

(Slide 18)

We know very well that cigarette smoking has a profound affect on the developmental biology of the fetus. It produces low birth weight, increased risk of inter-uterine growth retardation; and we know from a variety of epidemiological studies, some of which have followed cohorts for over 40 years from the time of their birth, that birth weight is associated with the occurrence of asthma, the occurrence of wheezing episodes, abnormal respiratory mechanics at the time of birth, and the occurrence and mortality from chronic obstructive lung disease in adult life, and also generally lower levels of lung function.

So, birth weight in a variety of studies has been an important link between things that happened early in life and subsequent risks.

You may be aware there have been a series of studies that have appeared in the last several years about the effects of exposure to environmental air pollution, criteria air pollutants, and effects on various pregnancy outcomes.

(Slide 19)

This is a study which appeared fairly recently from the Czech Republic which looked at exposure to PM-10 during each of nine months of pregnancy. I think there were about a thousand women who were looked at in this study.

What they did is look at the odds ratio of evidence of inter-uterine growth retardation, which is less than a tenth percentile weight for gestational age. What they basically found was that the largest effects -- here is shown the median exposure (40 to <50 mcg) and high exposure (>50 mcg) -- were in the first trimester.

Now if you remember when I showed you that little chart, it's in the first trimester that the airway system, the conducting system is laid down. It's pretty much finished by that period of time. Now in fairness, there are some other studies that have looked at this problem and have found -- from different environments, particularly in China, that the effects might be later in pregnancy. But this is certainly an intriguing finding.

There are also studies which have now shown that exposure during pregnancy is associated with post-neonatal respiratory morbidity and mortality, as well as increased fetal loss after the 28th week of pregnancy.

So, there's a developing body of data that these exposures potentially are having effect on fetal development.

(Slide 20)

This is just to show you the effect of birth weight in prematurity -- this is from some work that Kathleen Mortimer, who's now at U.C. Berkeley, conducted as part of her dissertation -- from the National Cooperative Inner City Asthma Study. She was trying to identify which subgroup of asthmatics were particularly responsive to daily changes in air pollutants.

This is a daily time series study, and looks at responses, decrements in peak expiratory flow, and the incidence of morning symptom to a 15-PPB increase -- this is actually I think a three-day moving

average. You will notice, here's the results for normal birth weight, full-term infants, and these are infants who are either low birth weight or premature, that is, less than 36-week gestation. You can see both in terms of the magnitude of the effect and the odds of symptoms that it is in the low birth weight group where the responses in her dataset were occurring. There was little evidence that the normal birth weight babies were responding at all.

(Slide 21)

One of the things that's been known for a long time in adults is that the level of pulmonary function, usually measures of airways, is an important predictor of this trait called airways reactivity. We just finished a study that was actually part of a study of the effects of environmental tobacco smoke and viral infections of 154 infants who we studied about 180 days of life. After we corrected for all the things that affect their level of this flow measure and that could indirectly affect their measure of airways reactivity (which we measured directly), it turns out that their lung function at the time we studied them was still a predictor of this trait which we know is a strong predictor for the occurrence of asthma -- as I'll show you -- and a strong predictor for the growth of lung function over the life of a child.

(Omit slide 22)

(Slide 23)

This is looking at a study that involved a large number of children that I was involved in that were followed for about 13 years. This was a longitudinal study in which we looked at various factors that affected the growth of the lungs between age five and age 20. We have identified those factors here, which after you correct for the somatic growth of the child, their lung function, their previous measurements, which adversely affect the growth of their lungs over the period of middle childhood through adolescence. You will notice current smoking, even though these kids didn't smoke very much, exposure to maternal smoking, and whether or not these kids had hyperactive airways affect their level of lung function at each observation. So this trait (these children who were not asthmatic and not ill) is a marker for alteration in the growth of the respiratory system in children.

(Slide 24)

Similarly, a study that was done in Italy on about 200-odd childrenthat evaluated the risk of asthma based upon their response to a test at age three years of age. This is sort of a life table analysis. You'll notice that those children who were responding to this particular test who had been asymptomatic before age three, had a much higher risk of subsequently developing asthma. So this, again, to illustrate the potential value of this trait.

(omit slide 25)

(Slide 26)

This is another recent study that was carried out in one of the Scandinavian countries, I don't remember where, looking at the effect of atopic status. Atopic status, atopy is sort of a term that had a clinical origin and has been co-opted by epidemiologists. Probably the simplest way to describe it is the signs and symptoms of allergic response that are mediated specifically by the part of the immune system that produces IGE antibodies. It is measured by things like asthma, eczema in childhood, hay fever, and in epidemiologic studies by responses to skin tests. The reason I include this is this was a study looking at longitudinal study of factors that predicted the level of this particular measure of lung function at age 12 to 19 years. The point I want to make here is after taking into account a bunch of other things, the acquisition of new allergy in the past -- in other words, they studied these kids on two separate occasions and then looked at what happened to them over these, this age group -- and the acquisition of this new allergy was negatively related to the subsequent development of the respiratory system during their teen years.

(Slide 27)

Again to relate this to the morbidity, this is a study looking at a large number of children who had one or more parents with atopic disease and, therefore, at relatively high risk based on familial history. It looked at factors that predicted whether these children would wheeze in the first year of life. The study was looking at a variety of things: the presence of cockroach antigen in the dust, having a dog in the home, etc. These measurements were made prior to the time the children were sick. The measurements were made right after the children came home, within a few months, and then the children were observed.

These exposures are clearly having affects on symptoms. If you think about what I said before, that people who are symptomatic very frequently have these functional alterations before they ever got symptomatic, and you can begin to see how some of these things come together.

Well, I want to step out, away from the respiratory system, per se, for just a minute and try to bring all of this together in some way that ties these sort of anatomical, mechanical characteristics that clearly appear to be influenced by things like environmental tobacco smoke, and probably are going to turn out to be shown to be influenced by exposure to ambient air pollution at critical development points. Let's look for a minute at the development of immunologic competence, if you will, in the newborn, and how it may be related to some of these environmental exposures that (as I indicated when I talked about atopy) impact on the symptom burden and on the growth of the respiratory system.

I now want to look at one of the contemporary hypotheses about how atopy may express itself in people who are genetically predisposed. and how the environment may potentially enhance the expression of this and feed into this entire system.

(Slide 28)

Here are some data that look at a group of children who were studied from birth to two years. And without getting into the details too much, let's just say that this particular cytokine here, interleukin-4, is a

marker for the allergic propensity as I've defined it, and this marker here, interferon-gamma, is a marker for what we'll call the non-allergic phenotype.

The important thing to note here is that at two years of age, if you look at what's happening with this marker here, the ones who are destined to become -- these are non-atopic and here are the ones that are atopic. You'll notice in the non-atopic that starting from birth out to 18 months is a progressive decline in IL-4 and a very sharp increase around six months of age in the production of this marker of immune maturation (gamma interferon). Whereas, in the children who are destined to become atopic you'll notice that IL-4 declines very slightly and then rises again by 18 months, and that there doesn't appear to be the sharp peak in gamma interferon which has only a very slow rise.

Now, how does this relate to what goes on in the fetus? That's the next slide. I'm not going to go through this in detail.

(Slide 29)

This thing labeled here TH-2 we'll call the allergic phenotype. It turns out that the pregnancy state is a TH-2 state. That is, when you're *in utero*, the sort of response milieu of these modulators of a variety of inflammatory and immune responses is skewed towards this, what when you're an adult would be characterized as the, if you will, the allergic phenotype, and is skewed away from this, we'll call non-allergic phenotype, TH-1.

It's now thought that the critical events in terms of determining whether a child, given a certain genetic risk, becomes allergic may have to do with a occurrence of when this immune deviation to the more -- the non-allergic phenotype takes place.

(Slide 30)

I have written here sort of the ontogeny of atopy, the failure of immune deviation, which is the hypothesis that initial priming of the cells responsible for atopic immune response, occurs to antigens to which the mother is exposed in the third trimester. In other words, the fetus is responding *in utero*.

This TH-2 we called the atopic phenotype. The non-atopic infants show this age-dependent decline, which I showed you before, which is not seen in persons who become atopic, and it's the failure of this immune deviation -- that is, that age-related shift from this allergic *in utero* skewed phenotype to the more non-allergic phenotype which tends to be most noticeable in the infants who are at greatest genetic risk for atopy.

Therefore, environmental factors that inhibit immune deviation could enhance the genetic risk of atopy. Since atopy is such a strong predictor of both childhood respiratory morbidity and adult respiratory morbidity, it's worth looking at.

(Slide 31)

Now one of the areas that's really interesting has been some of the immunologic phenomena that have been associated with the exposure to diesel exhaust particles. I briefly summarized them here, that in already-sensitized subjects exposure to diesel exhaust particles has been associated with an increase in the

antibodies that are associated with allergy, as I've defined it, to mucosal antigens, increase in the number of the cells that produce these antibodies, actually switching of cells to the production of these antibodies, and a skewing of this chemical environment to what we called the allergic phenotype, and an inhibition of the production of the markers that in essence mark immune deviation towards a non-allergic phenotype.

In fact, you can show that this skewing of the immune response occurs in individuals who are not necessarily sensitized in advance.

(Omit slide 32)

So here's a situation where we look at what we now are beginning to know about the development of the response -- one of the major ways that the respiratory system responds to the environment, there is reason to think that the environment is in fact potentially influencing that at a very critical point, and may be reasonable for determining whether a genetic predisposition is actually expressed.

(Slide 33)

So in conclusion, I think it's fair -- we can say, I think fairly, that environmental exposures that occur during fetal life and during the first few years of life have been shown, at least in my mind, pretty clearly to affect lung function development. There are subtle alterations in the growth of the lung and its attendant mechanical -- it should say "mechanical" "properties" -- underlying risk factors for the occurrence of childhood respiratory disease and the severity of respiratory disease. And I think that's been pretty well established.

(Slide 34)

Then finally, alterations in lung function influence the occurrence and the severity of this trait called airways reactivity. As I showed you, this occurs very early in life. And this trait itself is a marker for altered lung function both in childhood and adult life. Children who are atopic have slower lung function development, and atopy is the major source of the pulmonary inflammatory reaction that characterizes childhood asthma. Finally, the development of the atopic state may be enhanced by non-allergen environmental exposures, and such exposures may, in turn, enhance the exposure allergin.

So I think in answer to the questions put before me, I think we need to spend a lot more time understanding the interface between fetal development of the respiratory and immune systems and environmental exposures if we're really going to understand a lot of the morbidity of childhood respiratory disease. Thank you. (Applause.)

DR. MARTY: We'll take one question maybe, or two. We have one question.

DR. DEMPSEY: Delia Dempsey from San Francisco.
First a comment, that although a lot of people use maternal smoking during pregnancy as a marker for -- you know, and leave it at that, women don't quit smoking when the baby is born, so those babies are going

to be exposed postpartum, and that's something to remember. And the peak incidence of smoking, or prevalence of smoking in this country was about 20 years ago, when it was about 45% of the population, and since that time it has declined to about 23 to 25% of the population, and yet our asthma levels in children have risen. How do you explain that? I mean, and I do support that I think smoking does affect lung development, so I'm not calling that into question. But, how do you explain this sort of like paradox of rising asthma levels with probably a decrease in prenatal exposure to cigarette smoking?

DR. TAGER: Okay. First of all let me just make clear, the reason I presented the data on environmental tobacco smoke was not to make the case that it's the preeminent cause of asthma, it was to -- it's the exposure for which we have the best data that we can link with development. So that's the first -- sort of by way of disclaimer. The answer to your question simply is I don't know. I mean, I could offer you a number of theories which any number of people could come up with. I don't think that we know what the answer -- I certainly don't know what the answer is. I think there are a lot of interesting hypotheses for environmental exposures that range all the way from changing the housing stock, and temperature and humidity and indoor antigens, to qualitative changes in air pollutants as we move from industrial sources of pollution to vehicular and diesel sources, and whatever. For example, these immunologic responses, I don't know what the answer is. There are a lot of interesting things to study. But I don't -- somebody else may have a more definitive view, but I certainly am keeping my mind open.